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Studies on the Apple Rust Caused by *Gymnosporangium Yamadae* Miyabe.

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STUDIES ON THE APPLE RUST CAUSED BY *GYMNOSPORANGIUM YAMADAE* MIYABE.

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(With Plates XVII—XX.)

1. Introduction.

The genus Gymnosporangium is composed of a group of heteroecious fungi having but one exceptional species. The mycelium of one phase, namely the teleutostage, exerts a stimulating action on its coniferous host, causing the deformities of stem or leaf in various forms. Some species may cause the formation of small spherical galls on the twig, and some bring about the abnormal enlargement of the stem of the host plant, while others lead to the development of witches-brooms.

According to Kern (*21*), and Sydow (*33*), *Gymnosporangium globosum* Farlow, *G. Nelsoni* Arth., *G. corniculans* Kern, *G. floriforme* Thaxt., *G. Juniperi-virginianae* Schw., and *G. bermudianum* (Farl.) Earle cause the globular excrescences on Juniperus in America. No European species seem to induce such a spherical gall. The fungus under consideration also makes its appearance on the small twigs of *Juniperus chinensis* L. and *J. Sargentii* Takeda in Japan as a tiny spherical gall among the leaves. According to Prof. Miyabe (*26*), *Malus pumila* Mill. var. *domestica* C. K. Schn., *M. Sieboldii* Rehd. and *M. Halliana* Koehne serve as the hosts for the aecidial stage of the fungus. When the aecidial stage of the fungus occurs on the leaves of the cultivated apple tree, the name "Aka-boshi-byo" (Red spot disease) (*15, 16, 17, 25, 43*,), or "Ke-sabi-byo" (Hairy rust disease) (*13*) is used to designate the disease. The disease causes premature defoliation and a reduction in the vital activities of the less seriously affected leaves and consequently reduces the vigor of the apple trees. The young twigs may also become affected and die. Thus diminution in crops and deformation of the affected fruits cause a serious

loss to the apple growers.

It seems that this disease is distributed throughout the northeastern part of Japan and is especially abundant in the prefecture of Aomori. The abundance of the disease in that locality is due in part to the general practice of planting juniper trees as ornamental trees in gardens. The apple rust fungus probably had existed in that district for centuries, where the wild crab apple is found common before it found a new host in the apple tree when it was imported there. Ideta (*16*) states that the apple rust occurs in Kanazawa. It may be the southernmost locality for this disease.

In the following chapters the results of certain investigations will be presented, with reference to the relative susceptibility of apple varieties to the rust, to the conditions of spore germination of the causal fungus, and to the anatomy of the deformities brought about by the fungus.

The writer wishes to express here his sincere gratitude to Prof. K. Miyabe, at whose suggestion the work was begun and under whose supervision it has been carried out, and to Dr. G. Yamada, Prof. S. Ito, Prof. T. Hemmi and Mr. Z. Shima, to whom he is also indebted for their valuable suggestions.

2. Symptoms.

a) On apple trees.

The rust makes its appearance first on the upper surface of the leaves as very small greenish yellow spots about one mm. in diameter. These spots begin to show in one or two weeks after the infection has taken place. They enlarge gradually and assume an orange-yellow color often bordered by red concentric rings. In the lesions minute yellow pustules soon appear, which vary in number according to the size of the affected area. These pustules represent the openings of the spermogonia which exude droplets of a substance attractive to insects and soon afterward turn black. Spermogonia are produced not only on the upper surface of the leaves but sometimes on the under side of the leaves, as well as on the petioles. When the infection occurs on the under side of the leaf, the midrib and veins alone are attacked, often causing the affected portions to become swollen to double their normal size and causing the leaves to curl. The affected petiole assumes a spindle shape of orange color. The leaf tissues beneath the spermogonia become hyperplasied forming blisters

from one half to one centimetre in diameter. About 40 days after the appearance of spermogonia, aecidia are produced on the under surface of the hyperplasied portion of the leaf blade, on the swollen veins, midribs, and petioles. Aecidia may be arranged in a circle near the margin of the swollen area or may be scattered over the lesion. When aecidia are young, they appear as minute tubular projections, but when mature, the wall of the tube or pseudoperidia may split into a lace-like netting and the brown aecidiospores therein may be dispersed. Rarely the aecidia are produced on the upper surface of the leaves. In such a case, however, they are confined to the midrib near the petiole. In severe cases the leaves may turn yellow and fall before the aecidia appear. When a large number of infections have occurred on a leaf, the spots are generally small and they often coalesce with each other. Young twigs also may be attacked, resulting in the formation of short and stubby twigs, on which both spermogonia and aecidia may be produced in abundance. Seriously affected twigs may die at the end of the season. Infested fruits have been found, often deformed and dwarfed, in Rawle's Jannet. The lesions on the fruits are similar to those on the leaves except for their larger size and number of aecidia. At first the lesions are yellow but afterward turn dark brown and become wrinkled. The lesions on the fruits seem generally to be confined to the blossom end. The symptoms on *Malus Sieboldii* and *M. Halliana* are almost identical with those above mentioned.

b) On juniper trees.

The first evidence of infection on *Juniperus chinensis* L. and *J. Sargentii* Takeda by the fungus is visible in late summer as a small green excrescence on the side of the twig, originating at the base of a leaf. The affected part enlarges rapidly and reaches its full size during the winter, when the gall becomes brown, from globose to reniform in shape, and of a diameter varying from one-fifth to one centimeter. In the following May (in the latitude of Sapporo), after the rupture of the outer bark, the teleutosori appear in the form of cushions of a dark brown color. During rains they become gelatinized and swell up to twice or three times their original size; while the teleutospores embedded in a gelatinous matrix begin to germinate freely producing promycelia and discharging sporidia, which may be carried away by the wind and produce infections, if they happen to fall on the leaves of apple trees. Later the galls die, but often they remain attached to the twig for a long time.

3. Causal fungus.

The apple-rust fungus was first named *Gymnosporangium Yamadae* by Prof. Miyabe in 1903. He (*26*) states that the fungus, in the aecidial stage, attacks the leaves of *Malus pumila* Mill. var. *domestica* C. K. Schn., *M. Sieboldii* Rehd. and *M. Halliana* Koehne and that it causes, in the teleutostage, the globular galls on the stem of *Juniperus chinensis* L. The original description was presented with the specimens of the fungus to the Anglo-Japanese Exhibition in London, 1909. It is as follows:—

Gymnosporangium Yamadae Miyabe

I. Pseudoperidia on slightly thickened reddish brown spots, cylindrical, 5–8 mm. long, 0.4–0.5 mm. in diam., becoming loosened into fine lace-like network, light brown. Peridial cells smooth on the inner side, slightly warted at one end; markings short rod-shaped, and not mingled with longer lines; 60–80 × 20–24 μ. Aecidiospores subglobose, polygonal, thick-walled, brown, finely punctate, with about 8 scattered germ-pores, 16–24 μ.

III. Teleutospore masses formed in globular galls, rupturing the rind in irregular streaks; when swollen compressed, petaloid, or tongue-shaped. Spores two-celled, oblong-clavate, broadly elliptical or obovate-elliptical, upper cell always larger and sometimes produced into a thick blunt papilla, 40–50 × 15–22 μ.

I. On the leaves, branches and fruits of *Pyrus Malus*, *Toringo*, *spectabilis*, and *baccata*.

III. On the branches of *Juniperus chinensis*. Japan: from Hokkaido to Middle Japan.

Two years later Prof. Miyabe published the description of the fungus in Japanese in Ideta's Hand-Book of the Plant-Diseases of Japan (*16*). In the same year, Kern (*19*) gave a description of the aecidial stage of the fungus on *Pyrus spectabilis* under the name, *Gym. Yamadae* Miyabe, and Sydow (*35*) later, in 1914, described the teleutostage under the same name. In 1914 Long (*23*) found a new fungus which he named *Gymnosporangium chinensis*, on *Juniperus chinensis* imported from Japan into America. The identity of *Gym. chinensis* Long with *Gym. Yamadae* Miyabe was proposed by S. Ito (*17*), but not definitely established. On the other hand, Clinton (*4*), Jackson (*18*), and Kern (*20*) considered Long's new species to be identical with *Gym. Haraeanum* Syd., (*Gym.*

asiaticum Miyabe) the pear rust fungus in Japan. Recently the literature of this subject was fully reviewed by Tanaka (*36*).

4. Relative susceptibility of apple varieties.

a) Results of inoculation experiments.

Some apple varieties are reported by Reed and Crabill (*28*), Gidding (*11*), and others* to be resistant or immune to the rust caused by *Gymnosporangium Juniperi-virginianae* in America. To determine the relative susceptibility of different apple varieties to the rust caused by *Gymnosporangium Yamadae*, a large number of inoculation experiments were carried out in the seasons of 1918, 1919 and 1920. The spore masses obtained from the galls on the twigs of *Juniperus chinensis* were sown in water. They became swollen into yellow gelatinous masses and the spores began to germinate readily. When the germination of the spores was ascertained, the gelatinous spore masses were smeared on the apple leaves. Then each of the inoculated plants was covered with a bell glass for a day so that the moisture would be retained. During the period of experiments several juniper plants bearing galls were kept in-doors to facilitate the inoculation at the desired moment.

Besides *Malus prunifolia* Borkh. var. *Rinki* Rehd. and *M. baccata* var. *mandshurica* Schn., fifty varieties of cultivated apples were used for inoculation. They were mostly one year old grafted plants. The results of experiments are presented in the following tables.

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* Jones, L. R. and E. T. Bartholomew, Apple rust and its control in Wisconsin. Wisc. Agr. Exp. Sta. Bul. 257. 30 p. 1915.

Pammel, L. H., The cedar apple fungi and apple rust in Iowa. Ia. Agr. Exp. Sta. Bul. 84. 36 p. 1905.

Table 1. Results of inoculation experiments in 1918.

Name of varieties	Date of inocul.	Results of inoculation		Remarks
		spermogonia	aecidia	
Red Astrachan	May 22	June 4	July 15	lesions large, hyperplasied
Prince of Bismarck	"	June 5	July 23	"
Ortley	"	June 6	July 22	"
American Summer Pearmain	June 8	June 17	July 27	"
Jonathan	"	June 18	July 26	"
Summer Queen	"	"	July 27	"
Red Astrachan	"	"	"	"
Peach	"	"	"	"
Dominey	"	"	"	"
M. Prun. var. *Rinki*	"	"	"	"
Rawle's Jannet	"	"	"	"
McIntosh Red	"	"	No aecidia	lesions minute, not hyperplasied
" "	June 12	June 19	"	"
American Summer Pearmain	"	"	July 26	lesions large, hyperplasied
M. Prun. var. *Rinki*	"	"	July 27	"
Maidens Blush	"	"	July 28	"
Duchess of Oldenburg	"	"	"	"
Rawle's Jannet	"	"	"	"
Swaar	"	"	entirely defoliated owing to severity of the rust	"
Tallmans Sweet	June 25	July 1	Aug. 5	"
Carolina Red June	"	"	defoliated	"
Williams Favorite	"	"	"	"
Whitney	July 5	....	....	no infection, probably owing to the late inoc.
American Summer Pearmain	"	....	....	"

Table 2. Results of inoculation experiments in 1919.

Name of varieties	Date of inocul.	Results of inoculation		Remarks
		spermogonia	aecidia	
St. Lawrence	June 6	June 15	July 28	lesions large, hyperplasied
Northern Spy	"	June 17	July 26	"
Yellow Transparent	"	"	Aug. 1	"
Porter	"	"	defoliated	"
Baldwin	"	"	Aug. 1	"
Ben Davis	"	June 19	Aug. 7	"
McIntosh Red	June 7	June 20	no aecidia	lesions minute, not hyperplasied
Alexander	"	June 17	Aug. 7	lesions large, hyperplasied
Wealthy	"	"	"	"
Alexander	June 16	June 30	"	"
Black Ben Davis	"	June 25	"	"
Newtown Pippin	"	"	"	"
Early Harvest	"	"	Aug. 1	"
McIntosh Red	"	"	no aecidia	lesions minute not hyperplasied
" "	"	"	"	"
" "	"	"	"	"
Smith Cider	"	June 26	Aug. 7	lesions large, hyperplasied
Tompkins King	"	"	defoliated	"
Koshojo	"	June 29	"	"
Cogswell ?	"	June 28	"	"
"	"	June 30	"	"
Gravenstein	"	"	"	"
* Fameuse	June 7	June 17	no aecidia	lesions minute, not hyperplasied
* McIntosh Red	"	June 18	"	"
* Red Astrachan	"	"	Aug. 10	lesions large, hyperplasied

* Cuttings in the water culture.

Table 3. Results of inoculation experiments in 1920.

Name of varieties	Date of inocul.	Results of inoculation		Remarks
		spermogonia	aecidia	
Fameuse	June 11	June 22	no aecidia	lesions minute, not hyperplasied
McIntosh Red	"	"	"	"
M. baccata var. *mand.*	"	"	July 31	lesions large, hyperplasied
Basil the Great	"	June 21	July 24	"
"	"	"	"	"
St. Lawrence	"	"	July 26	"
Giant Jannet	"	"	July 22	"
West Field Seek no Further	June 14	June 22	July 24	"
Gravenstein	"	June 23	July 29	"
Twenty Ounce	"	"	defoliated	"
Porter	"	"	Aug. 2	"
Early Strawberry	"	June 22	July 26	"
"	"	"	"	"
Coopers Market	"	"	"	"
Fall Pippin	"	"	July 23	"
Fameuse	"	June 24	no aecidia	lesions minute, not hyperplasied
Fameuse sucre	"	"	defoliated	lesions large, hyperplasied
Konishiki	"	June 22	Aug. 1	"
Daikokuzukin	"	"	July 26	"
"	"	"	"	"
Mother	June 24	July 3	defoliated	"
Koshojo	"	July 11	"	"
Skinners Seedling	"	July 5	Aug. 13	"
Mansaku	"	"	"	"
Cogswell?	"	"	"	"
McIntosh Red	"	July 8	no aecidia	lesions minute, not hyperplasied
"	"	"	"	"

Name of varieties	Date of inocul.	Resnlts of inoculation		Remarks
		spermogonia	aecidia	
Hubbardston	June 24	July 5	Aug. 8	lesions large, hyperplasied
Whitney	"	"	"	"
Esopus Spitzenburg	"	"	"	"
" "	"	"	"	"
Fameuse	June 30	July 11	no aecidia	lesions minute, not hyperplasied
"	"	"	"	"

As the above tables show, McIntosh Red and Fameuse are remarkably resistant to the apple rust. The spots produced on the leaves of these varieties cease sooner or later to develop and remain 1/2–2/3 mm. in diameter for the entire season. Moreover only a few minute spermogonia are produced in the lesions, but no aecidia. Nine plants of McIntosh Red and 4 plants of Fameuse were inoculated and careful inspections failed to disclose the aecidia on them. In the season of 1919, cuttings of Fameuse, McIntosh Red and Red Astrachan in water culture were inoculated. Ten days after inoculation spermogonia were produced very abundantly on the leaves of Red Astrachan but less numerously on the other varieties. Fifty-four days later, aecidia appeared on Red Astrachan but on the leaves of Fameuse and McIntosh Red the lesions remained undeveloped producing no aecidia. It is interesting to note that McIntosh Red is known as the seedling of Fameuse (*2*), these two varieties being both resistant. But such is not the case for Fameuse sucre, a member of the so called Fameuse Group (*2*).

According to Mr. Shima of the Aomori Agr. Exp. Station, Fameuse and McIntosh Red are resistant to the rust in that locality. If the rust spots are produced, however, on the leaves of these varieties, they are as large as usual according to his observations in the orchards.

b) Anatomy of the affected leaves of susceptible and resistant varieties.

Prior to the formation of spermogonia on the leaf of the susceptible variety, the infected area can be discerned on account of its discoloration. Microtomed sections of such an area show the fungus mycelium running throughout the affected tissues. For the most part the mycelium is intercellular running downward along the walls of the palisade cells and then freely branching and extending among the spongy parenchyma cells.

Some hyphae grow closely appressed to the walls of the host cells, sending haustoria into the cell cavities. The most conspicuous things indicated in the affected tissue are the degenerating chloroplasts, which are more or less small in size and scarcely stained with Heidenhain's haematoxylin presenting a good contrast with the well stained chloroplasts in the intact cells. When spermogonia are produced, the mycelium collects under the epidermal cells which will be thrust aside and ruptured, several cells around the ostiole of the spermogonia being collapsed. The hyphae which contribute to the formation of the spermogonia interweave at certain points under the epidermis, forming a layer of pseudoparenchyma, from which arises a series of upwardly directed and parallel hyphae, the spermatiophores. The peripheral hyphae grow out to form the paraphyses which project through the ruptured epidermis. The spermogonia, thus formed, are flask-shaped structures measuring approximately 100–150 $\times$ 160–220 μ. The pseudoparenchyma consists of a compact mass of short celled hyphae with watery contents and provided with a single nucleus in each cell. The nuclei in the mycelium are small and round in shape, with a definite nucleolus and chromatin network. The spermatiophore is a narrow elongated cell with an elongated nucleus. The primordium of a spermogonium is a bud-like projection on the rounded apex of the spermatiophore. The projection contains finely granulated cytoplasm. When it has attained considerable size, one of the daughter nuclei, resulted from the nuclear division in the spermatiophore, migrates into the projected bud. The nucleus stretches out into a condensed bank-like mass to pass into the projection. The process of the nuclear division in the spermatiophore could not be definitely followed. The spermatium is, after having received a nucleus, cut off by abstriction from the spermatiophore. The nucleus in a spermatium is an almost homogeneous mass at first but soon becomes larger and shows a fine network. The mature spermatium is ovate to club-shaped and measures 4–5.5 $\times$ 9.5 μ. It contains a relatively large nucleus which shows a chromatin network but no nucleolus, a very small amount of cytoplasm and apparently no reserve material, the whole being enclosed in a very thin cell wall. The free spermatia accumulate in the cavity of the spermogonia and later become extruded through the ostiole. The affected tissues under the spermogonia become hyperplasied owing to an excessive enlargement and multiplication of the spongy parenchyma cells. The intercellular spaces of the spongy parenchyma are entirely obliterated and the cells become closely packed together giving the tissue a more compact appearance.

Those cells appear to be filled with starch grains and contain one somewhat enlarged nucleus usually presenting two nucleoli.

The first evidence of the aecidia formation is recognized by a conspicuous massing of entangled hyphae in the hyperplasied spongy parenchyma just beneath the palisade cells. The hyphal mass replaces the host cells which are gradually destroyed and eventually disappear. As the aecidium proceeds to develop, the hyphal mass, which is differentiating into peridial cells, basal cell, aecidiospore-initial cells, aecidiospores and intercalary cells, gradually enlarges and elongates towards the lower epidermis of the leaf. At length the aecidium ruptures the epidermis and protrudes as a brown papilla and then the peridium dehisces setting free the aecidiospores.

In the sections of the infected area on the leaf of McIntosh Red and Fameuse, it is found that the mycelial growth remains in the leaf tissue just under the spermogonia and does not extend so widely as in the normal infection. In the affected cells the nuclei are very distinct in the cell-lumen, as most of the chloroplasts are degenerated eventually, presenting a good contrast with the intact cells where the nuclei scarcely can be discerned among the well stained chloroplasts. The spermogonia are remarkably smaller than usual, measuring $75 \times 110\ \mu$ and produce a small number of spermatia. One occasion has been known, where the entangled hyphal mass was found in the slightly hyperplasied leaf tissue, indicating the early stage of the aecidium formation. Since the section was made from a leaf collected on September 12, the fungus possibly would be able to make no further progress for the entire season.

The relation between the rust fungus and plants highly resistant to its attack were first carefully investigated by Ward (*28*, *37*). He showed that when the brown rust of bromes, *Puccinia dispersa* Eriks. attacked its proper host, the parasite made a vigorous development without immediate serious injury to the host. Sometimes the host seemed not to be injured but somewhat stimulated. When infection took place on an uncongenial species of Bromus, as he pointed out, the fungus was found to kill some of the host cells very soon after gaining entrance and cause them to shrivel up as if corroded until the fungus itself could make no further progress. Ward (*39*) also examined the infection of wheat immune to the yellow rust caused by *Puccinia glumarum* Eriks. And he concludes that the so-called immune plants may be really badly infected, bearing innumerable minute yellow and brown spots, each of which is an abortive infection area; that the uredospores send germ tubes into the stomata, almost if not quite as frequently as they do into the susceptible wheat, but the walls of the

cells in contact with the hyphae turn brown and die and the hyphae are starved, extending not far from the point of infection.

Ward's histological work has been verified by Marryat (*24*) with *Puccinia glumarum* and immune and semi-immune wheat, by Gibson (*10*) with *Uredo Chrysanthemi* and many hosts widely separated taxonomically, and by Stakman (*30, 31*) with several biologic forms of *Puccinia graminis* and hosts both partially and highly resistant. Allen (*1*) also found a similar condition existing between *Puc. graminis tritici* and wheat immune to its attack. Marryat (*24*) states that "though the fungus succeeds in making its entry and producing hyphae, further progress is either completely checked by the breakdown and death of the host tissue locally, accompanied by the starvation and death of the parasite, in the immune host, or else a more protracted struggle takes place in the semi-immune host." In the latter case, as she writes, "the development of the fungus proceeds to a further point but is still greatly retarded as compared with a normal case." Such is the case between McIntosh Red or Fameuse and the apple-rust fungus as formerly mentioned. The problems as to the immunity of these apple varieties must remain unsolved, until further investigations proceed, though the theory that immunity is due to definite antagonistic chemical interactions between host and parasite is advanced by several investigators.

5. Germination experiments with the teleutospores.

a) The teleutospore and its germination.

The teleutospore is characteristically two celled but occasionally 3 to 5 celled spores are found. (Pl. XVII. Fig. 1). One celled spores occur but rarely. The spores range in width from 19 to 23 μ and in length from 35 to 70 μ; mostly 19 by 45 μ. The spore is broadly ellipsoidal or oblong clavate and slightly constricted at the septum. It has been known for a long time that there are two forms of teleutospores in different species of Gymnosporangium. In the teleutospores of the fungus under consideration, two forms of spores are also recognizable. The spores found in the inner portion of the spore-mass are symmetrical, having thin hyaline membrane, while the spores present on the outside are irregular in shape and have thick walls of dark brown color. For the most part the thick walled spores are two celled and stunted in their appearance while 3 to 5 celled spores are not infrequently found among the spores having the thin and hyaline membranes. The pedicels are hyaline, exceedingly

long and of equal diameter throughout. There are two germ-pores in each cell of the spore, one on each side of the cell near the septum and often in the upper cell one more pore can be found at the apex.

The germination is of the usual type, producing a promycelium which bears four sporidia. The mode of germination, however, is various, depending on the amount of the moisture and temperature, to which they are subjected. If supplied abundantly with water, it frequently happens that the germ tube grows out, instead of an ordinary promycelium, to a great length often more than ten times the length of the spore, the lower portion of the tube becoming nearly empty. The contents at the apical portion of the germ tube sometimes do not divide up, producing directly one or more thin tubes, but often become divided into 3 or 4 cells resembling the promycelium. Sometimes, as Blackman (*3*) has pointed out, these promycelial cells become rounded off themselves, separate from each other, and begin to germinate, producing a small tube. Various states of normal and abnormal germination of the teleutospores are shown in Pl. XVII. Fig. 2.

The sporidium is a small ovoid body measuring 7.5–9.5×13–$15.5\ \mu$. At a certain point on the cell wall is a tiny process which makes the point of attachment to the sterigma. The sporidium germinates by producing one, or rarely two thin germ tubes, the end of which bulges out in a rare case into a slight enlargement.

b) Results of experiments.

Materials. On January 8, 1919, some teleutosori began to appear on the twigs of *Juniperus chinensis* which had been placed in the greenhouse since the previous autumn. With the teleutospores thus obtained numerous germination tests were conducted during the following two months. In the late spring of 1920 similar germination experiments were carried out with the teleutospores produced under natural conditions.

The time required for germination. According to Heald (*14*) the promycelia and sporidia of *Gymnosporangium Juniperi-virginianae* Schw., the American apple rust fungus, will be produced in 12 to 24 hours under favorable conditions. Coons (*6*) states that the process of putting out germ tubes requires from 6 to 15 hours, for *Gym. Juniperi-virginianae*, in contrast to less than 3 hours as reported by Blackman (*3*) for *Gym. clavariaeforme*, while Weimer (*41*) found that from 3 to 4 hours are the usual time required for germination of the teleutospores of *Gym. Juniperi-*

virginianae under optimum conditions.

In hanging drop cultures, the teleutospores of *Gym. Yamadae* begin, at the end of one hour, to put forth germ tubes which grow one and half times the length of the spore in two hours and produce sporidia in about 4 hours at a temperature ranging from 18° to 20°C. These results coincide with those of Weimer (*41*) secured from germination experiments of the teleutospores of *Gym. Juniperi-virginianae*, although he made the spores germinate on slides in Petri dishes.

The influence of moisture on germination. The important factors affecting the germination of the spore are the amount of moisture and the temperature.

Blackman (*3*) found that the teleutospores of *Phragmidium Rubi* in hanging drops developed long germ tubes but no sporidia until they had grown barely to reach the air, while the spores suspended on the water in watch glasses produced the characteristic promycelia and sporidia at once. He concludes that the presence of free air is the determining factor for the sporidia formation. His theory seems to have been confirmed by Coons (*6*) who, working with *Gym. Juniperi-virginianae*, states that in air partly exhausted of oxygen by the pyrogallic acid method, the germ tubes of the teleutospores grew from 8 to 10 times the normal length but produced no sporidia and the spores about to germinate were prevented from germinating by an atmosphere of coal gas and carbon dioxide. Reed and Crabill (*28*) also failed to make the teleutospores and sporidia of the same fungus germinate in an atmosphere of carbon dioxide.

In the writer's experiments with the teleutospores of *Gym. Yamadae* the sporidia were produced as abundantly as the exceedingly elongated germ tubes in hanging drops. The spores suspended on the water in watch glasses yielded no better results, the spore masses being apt more or less to submerge into the water.

The influence of temperature on germination. Reed and Crabill (*28*) found 15°C. to be the optimum temperature for the germination of the teleutospores of *Gym. Juniperi-virginianae*, with 29° the maximum and about 11° the minimum. The upper thermal death point was 30°C.; the lower thermal death point was not determined, but was much lower than the freezing point of water. According to Weimer (*41*) the optimum temperature for the germination of the teleutospores of the same fungus ranges from 22° to 25°C, the best germination taking place at 23°–24°, while the lowest temperature at which germination occurred was 7° and the highest 29°C.

Numerous experiments have been conducted to determine the relation

of temperature to germination of the teleutospores of *Gym. Yamadae.* The spores in hanging drops were incubated at various degrees of temperature. The average results of these tests are shown in Tables 4 and 5.

Table 4. Teleutospore germination at various degrees of temperature. (Results of experiments from Jan. to Feb. 1919).

Temperature	Duration of incubation	Percent of germination	Remarks
7°C.	23 hours	0.5	no sporidia
10°—11°	"	10	
11°	"	20	
13°	"	65	
15°	"	97	
19.5°	"	97	
24°	24	93	no sporidia
26°	"	90	" "
28°	"	90	" "
30°	"	40	The spore put forth a small bud-like process which remained undeveloped.
30.5°	30	0	

Table 5. Teleutospore germination at various degrees of temperature. (Results of experiments from May to June, 1920).

Temperature	Duration of incubation	Percent of germination	Remarks
15°C.	15 hours	97	incipient germination, no sporidia
16°	"	97	
16°	24	98	
16°—17°	15	97	
19°	"	97	
20°	"	97	
22°	"	96	
24°	"	97	
24°—24.5°	"	95	no sporidia
24.5°	"	95	" "

Temperature	Duration of incubation	Percent of germination	Remarks
25°	15	95	no sporidia
25°	24	98	" "
25.5°	15	67	" "
27.5°	"	82	" "
30°	"	25	The spore put forth a small bud-like process which remained undeveloped.

The optimum temperature seems to range, as shown in the tables, from 15°C. to 25°C., the maximum being 30°C., and the minimum about 7°C. Some spore masses suspended in water had been frozen during 2 hours, and a majority of the spores put forth germ tubes very slowly after melting of the ice.

In the course of the experiments the writer's attention was called to the fact that no sporidia were produced at a high temperature, as shown in Tables 6 and 7.

Table 6. Influence of temperature on sporidia-formation. (Jan.–Feb., 1919).

Temperature	Duration of incubation	Formation of sporidia
7°C.	23 hours	–
11°	7	–
11°	23	+
13°	"	+
15°	10	+
20°	"	+
22°	7	+
23.5°–24°	22	–
25°	30	–
26°	25	–
28°	"	–
30°	"	–

Table 7. Influence of temperature on sporidia-formation.
(May-June, 1920).

Temperature	Duration of incubation	Formation of sporidia	Remarks
15° C.	15 hours	no sporidia	incipient germination
16°	"	abundant sporidia	
17°	"	"	
19°	"	"	
20°	"	"	
22°	"	"	
24°	"	a few sporidia	
24°—24.5°	"	no sporidia	incipient sporidia which remained undeveloped
25°	"	"	"
25°	24	"	"
25.5°	15	"	
27.5°	"	"	
30°	"	"	

Only a few sporidia were produced at 24°C. Above this degree of temperature the germ-tube lengthens out into a curled and vacuolated hypha branching abnormally. (Pl. XVII. Fig. 4) A similar case is known with the teleutospores of *Gym. Juniperi-virginianae* as reported by Reed and Crabill (*28*) who found that a few sporidia were produced at temperatures above 20°C., but none above 24°C. However, Weimer (*41*), working with the same fungus states that an abundance of sporidia were obtained at a temperature ranging from 22° to 25°C. and some sporidia were produced even at 26°C.

In the writer's experiments, it has been noticed that the teleutospores of *Gym. Yamadae* germinate more readily at a high temperature than at a low temperature, but no sporidia are produced in the former case: in other words, a low temperature retards the germination, while the germination at a high temperature is abnormal.

Therefore it may be concluded that the optimum temperature for germination of the teleutospores of the fungus under consideration ranges from 16° to 22°C.

Crabill (*7*) found secondary spores, which he designated as secondary

sporidia, on the germinating sporidia of *Gym. Juniperi-virginianae* as well as of *Gym. clavipes*. According to him the chief factor which affects the production of the secondary sporidia is an excess of moisture. In the sporidia of *Gym. Yamadae* such secondary sporidia could not be found either in the hanging-drop cultures or in the suspension of spores in water, though rarely the end of the germ-tube of the sporidia bulged out into a slight enlargement indicating similarity to the early stage of the secondary sporidia such as figured by Crabill.

Light seems to have no influence on the germination of the teleutospores of the present fungus as the spores in hanging-drop cultures kept in the dark differed in no respects from those germinated in the light.

6. Germination experiments with the aecidiospores.

a) The aecidiospore and its germination.

The aecidiospores of *Gym. Yamadae* are dark brown spherical cells measuring 23 μ by 19 μ. The spore possesses a thick brown, finely warted cell wall with 4 or 5 germ-pores distinctly visible at the time of germination. Germination takes place by means of a simple germ-tube which sometimes branches at the tip. (Pl. XVII. Fig. 5) The spore if sown in water, produces one (rarely two) small bud-like process, in 2-3 hours, which grows rapidly and reaches a length 20–50 times the diameter of the spore. The hyaline and granular contents of the spore pass into the germ-tube with growth, remaining always toward the distal end.

b) Results of experiments.

The fact that the aecidiospores of some species of *Gymnosporangium* could scarcely be induced to germinate has been known to many investigators. Heald (*14*) working with *Gym. Juniperi-virginianae*, states that after the first of October only one or two per cent of the aecidiospores were capable of germinating at outdoor, field temperature. Reed and Crabill (*28*) have experienced such a great difficulty in germinating the aecidiospores of the same fungus as to make them consider that the aecidiospore may overwinter in the axil of the cedar leaf and germinate in the following spring, giving rise to a young cedar gall. Weimer (*41*) insists that a small portion of the aecidiospores of *Gym. Juniperi-virginianae* and *Gym. globosum* germinated under artificial conditions.

The writer also has experienced great difficulty in germinating the aecidiospores of *Gym. Yamadae*. The results of the experiments are

shown in the following table:—

Table 8. Results of aecidiospore germination experiments in the summer of 1918.

Date	Temperature	Media	Method	Results	Remarks
July 27	28°C. ±	water	on slides	no germination	fresh spores
,,	,,	,,	in hanging drops	,,	,, ,,
,,	,,	juniper leaf juice	,,	,,	,, ,,
31	22°C. ±	water	,,	,,	,, ,,
Aug. 1	,,	agar	,,	,,	,, ,,
5	,,	water	,,	,,	,, ,,
Sept. 9	,,	,,	,,	,,	,, ,,
12	,,	water + juniper leaf	,,	,,	,, ,,
13	,,	water	on slides	,,	,, ,,
,,	,,	,,	in hanging drops	,,	,, ,,
16	,,	,,	,,	,,	spores collected on Aug. 10, 19 & Sept. 16
18	,,	,,	,,	,,	fresh spores
25	....	,,	,,	,,	,, ,,
28	....	,,	,,	,,	,, ,,
,,	....	water + juniper leaf	,,	,,	,, ,,
Oct. 1	....	water	,,	,,	spores collected on Sept. 21 & exposed to 0°C. for 10 days.
7	....	,,	,,	,,	fresh spores
14	....	,,	,,	,,	,, ,,
20	....	,,	,,	,,	spores collected on Sept. 17, 25 & Oct. 20

No germination of the aecidiospores was secured in water nor in the extracted juice of juniper leaf. Not only distilled water, but also double distilled water, tap water, and well water were used but no better results were obtained. Apple leaves bearing some aecidia were placed 10 days in a refrigerator where a temperature of 0°C. was prevailing. None of the spores thus treated germinated in water.

The inability of the aecidiospores to germinate under the conditions generally favorable for spore germination led the writer to the consideration that the aecidiospores of the apple rust fungus, as suggested by

Reed and Crabill (*28*), must undergo a rest period or exposure to winter conditions before they will germinate. Apple leaves bearing some aecidia were collected on September 25 and put into a cotton bag, which was tied to the twig of a small tree and left under the climatic conditions during the winter. On March 19 of the following spring, the bag was taken down and the aecidiospores therein were sown in hanging drops of distilled water. The germination tests were carried out in the greenhouse and the laboratory, where the temperature was about 23° and 18°C. respectively. After 3 hours some aecidiospores were found to have germinated both in the greenhouse and the laboratory. The germinating power was so vigorous that the germ-tubes had grown 17–20 times the diameter of the spore at the end of 20 hours, and about 65 % of the spores germinated in 24 hours. Some aecidiospores were sown in a hanging drop and observed constantly under the microscope. In one hour and a half three germinating spores were found and a large majority of the spores produced germ-tubes of a considerable length in three hours.

Some aecidiospores were kept in a Petri-dish and placed in a refrigerator where a temperature of 0°C. prevailed from Sept. 21 to March 27, a period of 187 days. The contents of those spores dried up, giving an angular appearance to the spores. None of those spores were capable of germination.

Table 9. Results of aecidiospore germination experiments in the spring of 1919, (hanging-drop cultures).

Date	Temperature	Media	Duration of incubation	Per cent of germination	Remarks
March 19–29	23°C.	distilled water	24 hours	65	spores collected on Sept. 25 and exposed to winter conditions
"	18°	"	"	"	"
20–21	"	"	38	60	"
"	"	"	48	0	spores coll. on Sept. 25 and kept in the room
27–28	"	"	"	0	spores coll. on Sept. 21 and kept in the refrigerator

In the summer of 1919, only a few aecidiospores of the apple rust fungus, *Gym. Yamadae*, could be induced to germinate, while those of *Gym. Haraeanum* Syd. (*G. asiaticum* Miyabe), the pear rust fungus, germinated readily under similar conditions. The results of the experiments are

shown in the following table:—

Table 10. Results of germination experiments with the aecidiospores of *G. Yamadae* and *G. Haraeanum* in the summer of 1919, (hanging-drop cultures).

Fungus	Date	Temperature	Media	Duration of incubation	Per cent of germination	Remarks
G. Yam.	Aug. 2–3	20°C.	dist. water	24 hours	0	fresh spores
„	„	20°(in the open)	„	„	0	„
G. Har.	„	22°	„	„	30	„
„	„	20°(in the open)	„	„	30	„
G. Yam.	2–4	22°	„	48	0	„
„	5–6	„	„	24	0	„
„	„	21°(in the open)	„	„	0	„
G. Har.	„	„	„	„	8	„
G. Yam.	7–8	23°	„	„	0	„
G. Har.	„	„	„	„	40	„
G. Yam.	11–12	„	„	16	0	„
„	„	„	juniper leaf juice	„	0	„
„	„	„	juniper leaf decoction agar	„	0	„
G. Har.	„	„	dist. water	„	55	„
G. Yam.	14–15	24°	„	„	7 germinated spores in a drop	„
„	„	„	5% saccharose solution	„	0	„
„	„	„	1% saccharose solution	„	0	„
„	„	„	5% glucose solution	„	0	„
„	„	„	1% glucose solution	„	0	„
„	15–16	„	dist. water	„	1 germinated spore in a drop	„
„	„	„	1% asparagin solution	„	0	„
„	29–30	20°(in the open)	dist. water	„	0	„
„	Sept. 2–4	....	„	40	0	„
G. Har.	„	....	„	„	5	„
G. Yam.	22–23	....	„	16	0	„

The experiments in the spring of 1920 to germinate the overwintered aecidiospores of *Gym. Yamadae* were unsuccessful. This was probably due to the fact that the spores, which were collected on Sept. 21 of the previous year, were placed so long in the room before being exposed to the natural out-of-door conditions that the contents of the spores dried up. The results of the experiments are as follows:—

Table 11. Results of the germination tests with overwintered aecidiospores of *Gym. Yamadae* in the spring of 1920.
(Hanging-drop cultures with distilled water)

Date	Temperature	Duration of incubation	Results	Remarks
Mar. 23	18°C. ±	24–28 hours	5 germinated spores in a drop	spores collected Sept. 21, kept in a cotton bag in the laboratry until Nov. 25, then exposed to out-door conditions.
25	"	"	0	
30	"	"	0	
Apr. 8	"	"	0	

In the summer of 1920, the aecidiospores of the apple-rust fungus, *Gym. Yamadae*, were found to germinate more readily after cooling in the refrigerator. The results of the experiments are shown in Table 12.

Table 12. Results of germination experiments with the aecidiospores of *Gym. Yamadae* and *Gym. Haraeanum* in the summer of 1920.

Fungus	Date	Temperature	Media	Method	Duration of incubation	Per cent of germination	Remarks
G. Har.	July 14	27.5°C.	dist. water	in hanging drops	17 hours	75	fresh spores
,,	27	26°	,,	,,	,,	55	,,
,,	Aug. 5	27.5°	,,	,,	15	53	,,
G. Yam.	,,	,,	,,	,,	,,	0	,,
,,	14	26°	,,	,,	21	0	spores placed on melting ice for one hour
,,	,,	,,	,,	,,	16	1 germinated spore in a drop	spores placed on melting ice for 4 hours
,,	,,	,,	,,	,,	,,	0	spores kept in a thermos flask at 9°–12°C. for 2 hours
,,	,,	,,	,,	,,	,,	10 germinated spores in 3 drops	fresh spores
,,	16	27.5°	tap water	,,	,,	0	,,
,,	,,	,,	juniper leaf decoct.	,,	,,	0	,,
G. Har.	,,	,,	,,	,,	,,	90–20	,,
G. Yam.	21	27°	tap water	6 drop cultures	,,	0	spores coll. the previous day and kept in the refrigerator at 0°C. for 1 day
,,	25	25°	,,	3 drops	,,	40 $\left(\frac{1285}{3165}\right)$	spores coll. on Aug. 20 and kept in the refrigerator (0°C) for 5 days
,,	26	26°	,,	,,	,,	0	fresh spores
,,	,,	,,	,,	,,	,,	2.4 $\left(\frac{39}{1627}\right)$	spores coll. on Aug. 20 & kept in the refrigerator for 5 days
,,	28	23°	,,	,,	24	0	fresh spores
,,	30	26°	,,	,,	16	$\frac{12}{556}$ in 1 of the drops	,,
,,	,,	,,	,,	4	,,	42 $\left(\frac{567}{1313}\right)$	spores coll. on Aug. 20 & kept in the refrigerator for 10 days
,,	Sept. 1	....	,,	6	,,	0	fresh spores
,,	8	21°	,,	3	,,	0	,,
,,	9	,,	,,	,,	,,	$\frac{54}{406}$	spores coll. on Aug. 20 & kept in the refrigerator for 20 days
,,	10	20°	,,	,,	,,	0	fresh spores
,,	,,	,,	,,	,,	,,	1 germinated spore in a drop	spores coll. on Aug. 20 & kept in the refrigerator for 20 days
,,	Oct. 5	15°	,,	5	,,	0	fresh spores
,,	,,	22.5°	,,	3	,,	0	,,

As the above table shows, the aecidiospores of *Gym. Yamadae* germinated more readily after cooling in the refrigerator at 0°C. from 5 days to 20 days. The percentage of germination was quite uncertain, varying from 3 to 60 in each of the drop cultures, according to circumstances, the cause of which remained unknown. It was found that the spores taken from one leaf germinated more readily than those from another, while the spores in one hanging-drop culture gave a higher percentage of germination than those in another. The germinating power was so vigorous that the germ tubes grew 20 to 30 times the diameter of the spore. It seemed that the pre-cooled spores readily lost their germinating power if they were left in the room more than one day after being taken out of the refrigerator.

In the early spring of 1921, the germinating power of overwintered aecidiospores of *Gym. Yamadae* was again tested. The results of experiments are shown in the following table.

Table 13. Results of germination testswi th overwintered aecidiospores of *Gym. Yamadae* in 1921.

Date	Temp.	Media	Number of hanging drops	Results	Remarks
Feb. 18	10°C.	dist. water	9	38 germinated spores in 8 drops	spores coll. on Sept. 10 & exposed to the winter conditions in the open
21	18°	"	5	20 germinated spores in 2 drops	"
23	"	"	"	0	spores coll. on Aug. 16 & overwintered in the open, the contents were dried up
24	"	"	8	0	spores coll. on Sept. 29 & overwintered in the open
Mar. 7	....	"	6	3 germinated spores in a drop	spores coll. on Aug. 26 & overwintered in the open
8	....	"	"	4 germinated spores in a drop	spores coll. on Sept. 10 & overwintered in the open

The percentage of germination, as the above table shows, is considerably lower than that secured in the spring of 1919. It can be stated, however, that the overwintered aecidiospores of the apple fungus, *Gym. Yamadae*, germinate more readily than the fresh spores.

As to the longevity of the aecidiospores of rust fungi there is much difference of opinion. De Bary considers that they may retain their germinating power for some weeks, while Plowright (27) states that "it is rather a matter of hours." "If the spores be placed in a very moist atmosphere they germinate at once; if, on the contrary, in a perfectly dry

one, they die very rapidly. But if they are kept slightly moist and cool, they will remain uninjured for a much longer time. I have seldom found them germinate after 48 hours and even then only a small portion will do so. It is only the mature spores at the top of the cup which will germinate." According to Bolley the spores of *Aecidium Rhamni* and *A. Berberidis* are capable of germination, even after being kept in the herbarium for some time or being sent through the post. Grove (*12*) writes, on the other hand, that the aecidiospores of *Puccinia Caricis* germinate when mature and fresh, but they may lose the power in a few days according to circumstances, especially being killed by rapid drying. According to Gibson (*10*), however, instances are known where the aecidiospores of *Phragmidium Rosae-alpinae* kept in a dry tube in a cool room from May 20 till July 28, a period of 69 days, germinated about one half, but every spore but one was dead after 82 days.

In the writer's experiments the aecidiospores of *Gym. Yamadae* retained their germinating power from Sept. 25 to March 20, a period of 177 days, or from Aug. 8 to March 7, a period of 212 days.

Concerning the conditions of the germination of aecidiospores there is some obscurity. Some previous investigators regard that the aecidiospores, which will not germinate in artificial cultures, are perfectly capable of producing infection on their own proper host. Eriksson found that the spores of *Aecidium Berberidis* were very uncertain in their germination, but there is always the possibility that spores may infect a host plant when sown on the living leaf, though they do not germinate in water. It has been ascertained by Klebahn (*22*) that the spores of *Peridermium Strobi*, which will not germinate on the *Ribes* decoction agar, readily infect the host when sown on the leaves of Ribes. The work of Spaulding on *Cronartium ribicola* (=*Peridermium Strobi*) also has shown that "spores which would not germinate at all in hanging drop cultures, on a water film, or on moist filter paper, either at room temperature or in an ice box or at room temperature after cooling, were perfectly capable of producing infection on species of Ribes." It was also found that "the aecidiospores frequently germinate—even then only a relatively low percentage of fresh spores do so—more readily after cooling in the ice box, and that sometimes they have to remain in the ice box to secure germination." (cited from Colley *5*)

The results of the writer's experiments show that neither juniper leaf juice nor sugar solution exerts any direct stimulus on the aecidiospores of *Gym. Yamadae*, while exposure to winter condition or cooling in the

refrigerator induces them to germinate more readily.

7. Anatomy of the gall.

a) Previous works on Gymnosporangium galls.

Sanford (*29*) was the first who investigated the anatomy of the spherical galls on *Juniperus virginianae* caused by *Gym. Juniperi-virginianae*. He considers the gall to be the result of an abnormal growth in the leaf tissues, which carries up the apex of the leaf as it develops. He further states that the vascular system enters the knot as one bundle given off from the vascular bundle of a branch and that the vascular bundle develops until it assumes equal importance with that of the branch and divides in a peculiar radiating manner throughout the knot, while the elements of the bundle are more distorted and less regularly placed. Wörnle (*42*) made an extensive study of the abnormal enlargement of the stem of host plants caused by three European and four American species of Gymnosporangium. According to him the galls induced by the European fungi, *Gym. Juniperinum*, *G. clavariaeforme* and *G. Sabinae*, arise from a swelling of the stems. The mycelium of *G. Juniperinum* is present in the wood as well as in the bast and cortex, and radially placed strands of parenchyma permeated with the mycelium are common in the wood. Wörnle considers the young shoots and buds to be only the passage through which the germ tube of the aecidiospore invades the wood tissue of Juniperus. He regards the cedar gall caused by the American fungi also as a hyperplasied twig. Reed and Crabill (*28*) have shown that the cedar apple is merely a hyperplasied cedar leaf, induced by *Gym. Juniperi-virginianae*. They give a diagramatic drawing of the vascular bundle of a young gall originating from the stem. Stewart (*32*) who has studied the histology of the galls caused by *Gym. Juniperi-virginianae* and *G. globosum* concludes that they arise from the axils of leaves and are transformed axillary buds, the leaf tissue also being involved in the gall formation. Weimer (*40*), on the other hand, makes the statement that these abnormal growths originate as modified leaves. The vascular systems of the galls, according to him, are composed of the enlarged and modified leaf trace bundles. Farlow (*9*), Wörnle (*42*), and Dodge (*8*) have contributed to the knowledge concerning the general distribution of the mycelium in the host tissue, in several species of Gymnosporangium.

In regard to the galls of *Juniperus chinensis* and *J. Sargenti* induced by *Gym. Yamadae*, nothing more has been known than that the gall

originates from the small twig of the host plants. In the early spring of 1919 the writer started work for further histological investigations of the juniper gall.

b) Methods.

The galls at various stages were fixed with Carnoy's fixative, embedded in paraffin and sectioned 15 microns thick with a microtome. Chromo-acetic fixative and Flemming's fluid were unsuitable owing to the disagreeable blackening of the material which it was difficult to bleach. Mostly Flemming's triple stains were used for staining the sections, while Pianeze's stain was found to be the best for demonstrating the intercellular mycelium. Safranin alone was used for the preparations to be photographed.

c) Structures of the juniper leaf and stem.

Before proceeding with the changes which result in the production of the gall, it seems desirable to note the structures of the normal leaf and young stem, since both of them are involved in the gall formation. The juniper leaf is attached tightly to the stem for a large part of its length, only the apical portion being free as shown in Pl. XVIII. Fig. 8. The epidermal layer on the upper surface of the leaf is provided with numerous stomata, while on the under side they may appear less numerous at the basal portion of the leaf. The normal leaf is triangular in cross section at the apex but becomes rather four-sided towards the base. The epidermis consists of a layer of somewhat flattened cells with heavily cutinized outer wall. Although the stomata occur mostly on the ventral epidermis in a cross section, they may be found on the dorsal side in a longitudinal section. (Pl. XVIII. Fig. 8) On the dorsal side of the leaf the epidermis is underlaid by a hypodermal layer. The interior of the leaf is made up of a parenchymatous tissue composed of loosely arranged large cells, closely resembling ordinary spongy leaf tissue. The outer layers of these cells are elongated perpendicular to the surface and form a palisade tissue. The central part of the leaf is occupied by a single vascular bundle of the collateral type, the xylem lying on the upper side and the phloem on the under. The xylem is composed of a small group of spiral and scalariform tracheids. On each side of the bundle there occur the so-called tracheidal parenchyma. Just beneath the vascular bundle near the base of the leaf is a resin canal. (Pl. XIX. Fig. 14)

In the young stem there is no boundary to be found between the stem and leaf tissue, as these tissues blend together at the point of union.

In Fig. 15, the leaf tissue is shown as three lobes connected with the stem tissue. The vascular system of the stem consists of a medullated central cylinder which is split up into several collateral bundles. A small group of pith cells occupies the center of the stem.

d) Structures of the gall.

The primodial gall makes its appearance in late summer as a small green excrescence on the side of the twig, which originates at the base of a leaf. The affected part enlarges rapidly and reaches its full size during the winter. Then the gall becomes brown, and is from globose to reniform in shape and of a diameter varying from one-fifth to one centimeter. The gall may be divided into four portions: the cork layers, the parenchyma, the vascular bundles and the fungus mycelium.

The formation of cork layers occurs at an early stage of the gall. Several layers of cork cells cover the gall tissue, surrounding also the stem where the gall is attached. (Pl. XVIII. Fig. 10) They are made of irregularly compressed dead cells. (Pl. XIX. Fig. 18) The cork layer is usually formed on the outside but sometimes another cork layer, so to speak, is found surrounding a compressed cavity formed in the peripheral parenchyma. (Pl. XIX. Fig. 18) Since such internal cork layers are found in extraordinarily large galls, they are considered to be the primary cork layers which once covered the gall tissues on the outside, but were broken out, thrust aside and consequently invaginated by the vigorous growth of the parenchyma, surrounded anew by the secondary cork layers. As a matter of fact some of the inner cork layers are connected with the outer cork layers. It will be of interest to note that the teleutosori sometimes occur underlying the inner cork layers instead of the outer cork layers, underside of which alone the sori are used to develop.

The parenchyma makes up the greater portion of the gall. It consists of somewhat elongated cells which are rather closely packed together, though there are quite large intercellular spaces among them. They are arranged with the long axes perpendicular to the stem. The parenchyma-cell contains a large nucleus presenting one or two nucleoli and is full of starch grains which will gradually disappear when the formation of teleutospores begins to take place.

Both the vascular bundles of the stem and the leaf trace bundle must be taken into consideration to interpret the origin of the vascular system of the gall, and to interpret it correctly is especially important for

determining the origin of the gall itself. Serial sections both longitudinal and transverse are absolutely necessary in order to avoid erroneous conclusions. Transverse sections of two young galls, one and a half mm. in diameter, are shown in Fig. 16 and 17 in Pl. XIX. It will be easily seen that the fungus mycelium which invaded the medullary rays of the stem has stimulated the elements to an enormous growth and accelerated division and consequently the splitting up of the xylem portion. (Pl. XIX. Figs. 16, 17, 19) Among the parenchymatous cells there can be found small fragments of the xylem portion in a semi-circular arrangement. The fungus mycelium runs through the intercellular spaces of the parenchyma, penetrating to the medullary rays. Both the gall and stem tissues are surrounded by layers of cork cells. Another section shows the two ends of the split xylem of the stem prolonging themselves to a considerable extent, giving the side view of the spiral and scalariform tracheids. Figs. 20, 21 and 22 in Pl. XX. show the transverse sections of a somewhat large gall of 1.5 × 2 mm., the sections being made of one and the same gall. In the gall of more advanced stage, the stem has become entirely enclosed by the gall tissue. A transverse section through the center of such a gall shows the xylem assuming a sickle shape, on the dorsal side of which the bast tissue develops. It is not uncommon to find the xylem cut off into several wedge-shaped fragments, as shown in Pl. XX. Fig. 23. Surrounding the central portion of the parenchyma, there are found distinctly the fragments of the vascular bundles in a circular arrangement. The strands of the small vascular bundles consist of phloem outside and xylem towards the centre, both of which are often much modified. More careful inspection discloses isolated and fragmentary tracheids extending from the stem and running through the gall tissue, while the sections of some sieve tubes can be found much apart from each other in the peripheral parenchyma.

Serial sections of a number of the galls in about the same stage of development have been examined, and always essentially the same structures are found. General aspects of the vascular bundles in transverse sections of the gall seem to show its origin to be from those of the stem and the basal portion of the leaf trace bundle, while what occupies the central portion of the gall is nothing but the broadened medullary ray cells and bast parenchyma cells of the stem with probably the basal portion of the leaf involved.

Now turning to the longitudinal sections of the gall, we find the sections of three young galls, two of which are 1.5 mm. in diameter and

the other measures 1.2×1.5 mm., as shown in Pl. XVIII. Figs. 9, 10 and 11 respectively. It will be clearly seen, that the vascular bundles have been derived from those of the stems, while the basal portion of the leaf trace bundle is involved in the gall. Undoubtedly the principal portion of the gall is made of the medullary ray cells and the bast parenchyma-cells, stimulated by the fungus to an enormous growth and accelerated division. In the section of a somewhat larger gall measuring 2×3 mm., as shown in Pl. XVIII. Fig. 12, the vascular bundle seems to be derived from that of the stem, though much bent and twisted. As shown in Pl. XVIII. Fig. 13, more highly magnified, the vascular bundle is remarkably distorted and in the small areas intervening between these twisted bundles the mycelium can be found. In Pl. XX. Fig. 24 which shows a section of a gall, 2 mm. in diameter, the vascular bundle of the gall is clearly seen to be derived from that of the stem. For the most part the hyphae run almost perpendicular to the vascular system of the stem. More careful inspection reveals fragmentary tracheids branching out of the vascular bundle of the stem at several points to extend into the gall. The sections of some sieve tubes often occur in the peripheral parenchyma. Fig. 25 in Pl. XX. shows a longitudinal section of a gall as large as 7 mm. in diameter. The strands of vascular bundles will be seen in concentric rings. In the outermost ring, fragments of the vascular bundle are arranged more uniformly, the phloem being outside and the xylem toward the centre, while the inner strands of the vascular bundle, which seem to be branched out of the outer vascular bundle, make a remarkably irregular arrangement. Sometimes there can be found distinctly the leaf trace bundle in a gall as large as 2 or 3 mm. in diameter, especially in a gall that points out at the tip towards the leaf which is attached to it. Such a bundle extends further upward to the base of the leaf and is often flanked on each side by the so-called tracheidal parenchyma which is to be found in the leaf. Thus there can be no doubt about it being a portion of the leaf trace bundle.

In short, the vascular bundle system of the gall is generally derived from that of the stem, though the lower portion of the leaf trace bundle is involved in the gall at the early stage of its development. The vascular bundle of the gall is composed of spiral and scalariform tracheids, on the outside accompanied with a few layers of elongated parenchyma-cells and sieve tubes. The elements of the vascular bundle are considerably modified.

The fungus mycelium is found throughout the gall tissues, occupying

the intercellular spaces in the parenchyma. It can invade every tissue except the cork cells and is found most abundantly just inside the cork layer, where afterward the hyphae become closely packed and interwoven together to form the teleutosorus. The mycelium is also found abundantly in patches along the medullary ray cells. The leaf tissue attached to the gall is also permeated with the mycelium. The mycelium, however, does not extend up and downward into the stem beyond the base of the gall. The galls attached to the stem, one just above the other, were sectioned, but no mycelium could be found in the stem between these two galls, showing that the infections were distinct and independent. The mycelium is septated and branches very frequently. The cells of the mycelium vary in their length and are binucleate. Haustoria may be occasionally found in the host cells. They are also binucleate.

e) The origin of the gall.

One of the most conspicuous things observable by the external examination of the gall is that there is always a discolored leaf-remain attached to each of the young galls. The leaf is attached to the gall with its base just in a similar manner as it would be with the stem. If such a leaf be removed, it is not unusual to find that the tip of the gall is pointed at beneath the leaf as shown in Fig. 12, which represents a longitudinal section of a gall measuring 2 × 3 mm.

It will be still further found that at the very early stage of the gall, it appears to break through the stem where the base of the leaf is attached. Furthermore, general aspects of the vascular bundles in transverse as well as longitudinal sections of the gall show, as before stated, that their origin is from the vascular bundle system of the stem and the basal portion of the leaf trace bundle, while the greater portion of the gall is composed of the hyperplasied medullary ray cells and bast parenchyma cells of the stem.

It is probable that the fungus gains entrance through the leaf, the mycelium later running down into the twig, since the leaf attached to the gall is found always to be permeated with the mycelium. The infection may take place on either side of the leaf, since the stomata often occur on the dorsal epidermis at the basal portion of the leaf, though they are for the most part found on its ventral surface. It will be easily seen, however, that at the axil of the leaf, which affords an easy lodgement for the spores and where the numerous stomata are found, there exists greater possibility of infection on the ventral surface of the leaf. When

the germ tube of an aecidiospore enters the leaf, it probably runs downward into the twig but not upward to the tip of the leaf, only the lower portion of it being permeated with the mycelium. Afterward, however, the dead leaf tissue may occasionally be found to be attacked by Alternaria and the other saprophytic fungi, the mycelium of which is dark brown and easily distinguishable from that of the fungus under consideration. The mycelium which has invaded the basal portion of the leaf trace bundle and then the vascular bundle of the stem, stimulates the bast parenchyma-cells and the medullary ray cells to an enormous growth and accelerated division resulting in the splitting up of the vascular bundle of the stem. The stem tissues at the base of the gall are greatly altered, while those at a little distance are still normal.

Thus the writer is inclined to believe that the gall originates from the stem tissues just beneath the base of the leaf on which the infection has occurred, the basal portion of the leaf trace bundle being also involved in the gall formation.

Summary

1. In the present paper, the morphology of the apple rust fungus, *Gymnosporangium Yamadae* Miyabe, the anatomical features of the gall induced by the fungus on *Juniperus chinensis* and *J. Sargenti*, and the results of inoculation experiments on 50 apple varieties are presented.

2. Of all 50 artificially inoculated apple varieties, Fameuse and McIntosh Red were found to be resistant to the rust. On the leaves of these two varieties, minute spermogonia were produced but the lesions remained undeveloped for the entire season producing no aecidia. It will be interesting to note that McIntosh Red is known to be a seedling plant of Fameuse.

3. The affected areas on the leaves of susceptible varieties hyperplasied owing to an excessive enlargement and mutiplication of the spongy parenchyma cells, which become closely packed together giving the tissue a more compact appearance. These cells appear to be filled with starch grains. The most conspicuous things apparent in the affected tissue are the degenerating chloroplasts which are more or less smaller in size and are scarcely stained with Heidenhain's haemotoxylin, presenting a fair contrast with the well-stained chloroplasts in the intact cell.

4. In the infected areas of the leaf of the resistant variety, the mycelial growth is restricted to the leaf tissue just under the spermogonia

and does not extend so widely as in the normal infection. In the affected cells the nuclei appear very distinct, as most of the chloroplasts have been degenerated, presenting a fair contrast with the normal cells where the nuclei can scarcely be discerned among the well-stained chloroplasts. The spermogonia are remarkably smaller than the ordinary ones produced on the leaves of susceptible varieties. They produce less numerous spermatia.

5. The optimum temperature for the germination of the teleutospores of the fungus ranges from 16°C. to 22°C., the maximum being 30°C. and the minimum about 7°C. A low temperature retards the germination, while the germination at a high temperature is abnormal, no sporidia being produced above 24°C. Above this point the germ tube lengthens into a curled and vacuolated hypha which branches irregularly. Light has no influence on germination.

6. The aecidiospores of the present apple rust fungus can scarcely be induced to germinate during the season in which they are produced, while those of *Gym. Haraeanum* Syd. (*G. asiaticum* Miyabe), the pear rust fungus, germinate readily under a similar condition. Neither the juniper-leaf juice nor a sugar solution exerts any direct stimulus on the aecidiospores of the apple rust fungus, while the exposure to winter conditions or cooling in the refrigerator induces them to germinate more readily. The aecidiospores of the fungus under consideration retained their germinating power from Sept. 25 to March 20, a period of 177 days, or from Aug. 8 to March 7, a period of 212 days.

7. The gall induced by the fungus on *Juniperus chinensis* originates as an abnormal growth of the stem tissue at the base of the leaf on which the infection has taken place, although the basal portion of the leaf trace bundle is also involved in the early stage of the development of gall. Several layers of cork cells cover the gall tissues surrounding also the stem where the gall is attached. The parenchyma which makes up the greater portion of the gall is made of the medullary ray cells and the bast parenchyma cells of the stem, stimulated by the fungus to an enormous growth and accelerated division. The gall consists of more or less elongated cells closely packed together and full of starch grains, which will gradually disappear when the teleutospore formation begins to take place. The vascular bundle system of the gall is in general derived from that of the stem, though the lower portion of the leaf trace bundle is involved in the gall at the early stage of its development. It is composed of spiral and scalariform tracheids, accompanied on the outside with a few layers of

elongated parenchyma cells and sieve tubes. The elements of the vascular bundle are considerably modified. The fungus mycelium is found throughout the gall tissue with the exception of the cork layers. The cells of the mycelium are all binucleate. The central cylinder of the stem is split up on the side next to the gall by the broadened medullary rays, which are permeated by the mycelium. The mycelium does not usually extend itself much upward or downward into the stem beyond the base of the gall.

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Explanation of Plates.

Plate XVII.

(Drawn with the aid of camera lucida)

Fig. 1. Teleutospores. (Zeiss $3 \times DD$)

Fig. 2. Germinating teleutospores and promycelia. ($4 \times DD$)

a. The apical portion of a germ tube which has grown out, instead of an ordinary promycelium, to a great extent.

b. The apical portion of the germ tube, divided into three or four cells, resembling the promycelial cells.

c. Two promycelial cells which have become rounded off themselves, separated from each other and begun to germinate.

Fig. 3. Sporidia and germinating sporidia. ($4 \times DD$)

Fig. 4. Teleutospores, germinated at a high temperature. ($4 \times DD$)

Fig. 5. Germinating aecidiospores. ($4 \times DD$)

Fig. 6. Juniper twigs bearing the galls induced by the apple rust fungus.

Fig. 7. *Ditto.*

Plate XVIII.

Fig. 8. Photomicrograph of a longitudinal section of a young juniper twig. *r.*, a resin canal.

Fig. 9. Photomicrograph of a longitudinal section of a young gall, 1.5 mm. in diameter.

Fig. 10. *Ditto*, showing *r.*, a resin canal.

Fig. 11. *Ditto*, the gall measuring 1.2×1.5 mm.

Fig. 12. Photomicrograph of a longitudinal section of a somewhat larger gall measuring 2×3 mm.

Fig. 13. The same section as above, more highly magnified.

Plate XIX

Fig. 14. Photomicrograph of a transverse section of the juniper leaf. *r.*, a resin canal, *st.*, stomata.

Fig. 15. Photomicrograph of a transverse section of the young juniper twig.

Fig. 16. Photomicrograph of a transverse section of a young gall, 1.5 mm. in diameter.

Fig. 17. *Ditto.*

Fig. 18. The internal cork layers.

Fig. 19. The same section as shown in Fig. 17, showing the central cylinder more highly magnified.

Plate XX.

Figs. 20, 21, 22.
Photomicrographs of transverse sections of a gall measuring 1.5×2 mm.

Fig. 23. Photomicrograph of a transverse section of a gall.

Fig. 24. Photomicrograph of a longitudinal section of a gall, 2 mm. in diameter.

Fig. 25. *Ditto*, the gall measuring 7 mm. in diameter.

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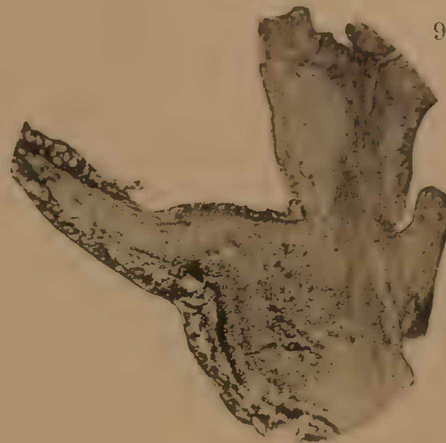

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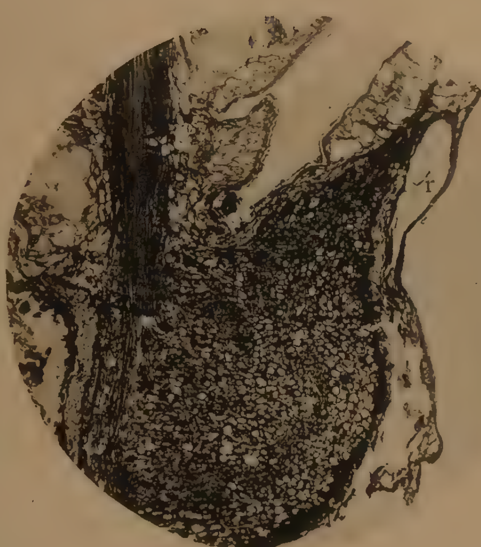

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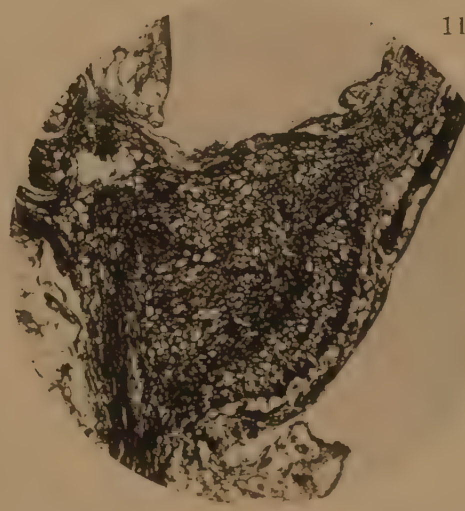

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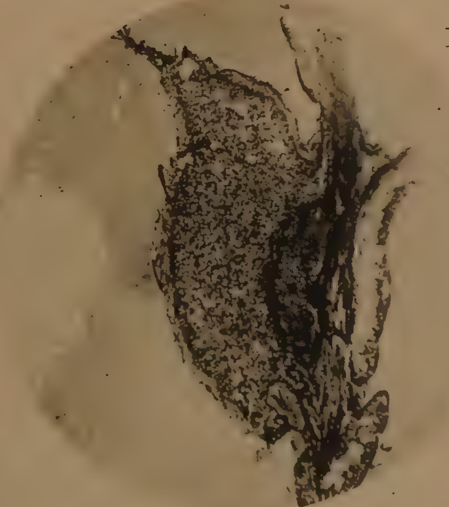

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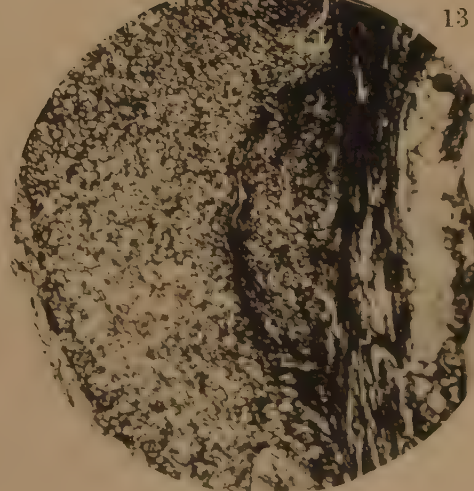

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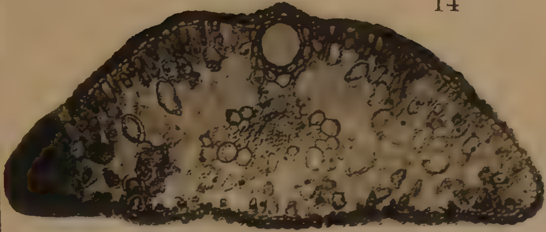

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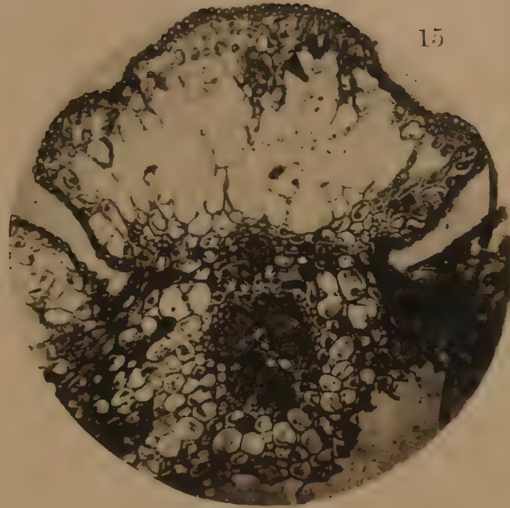

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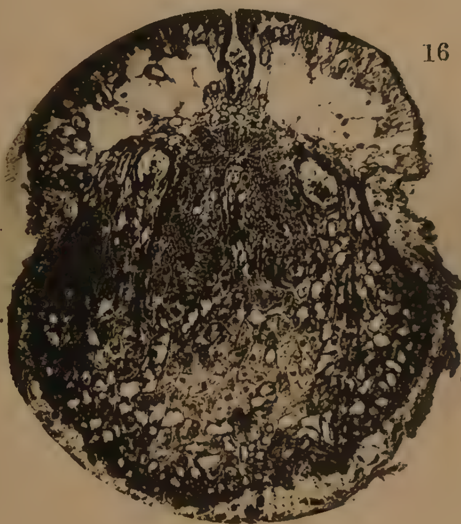

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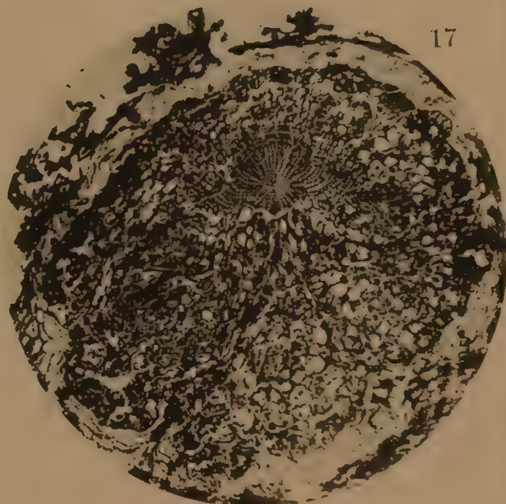

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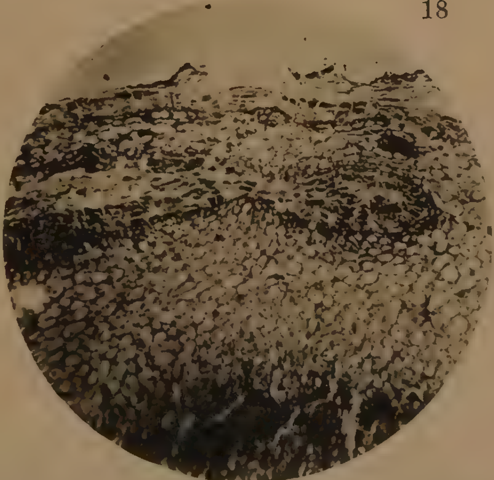

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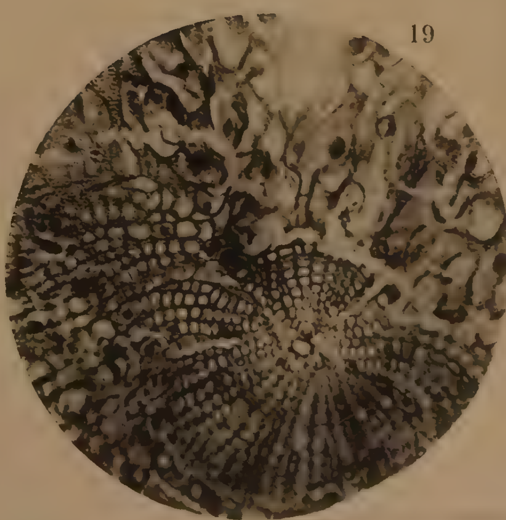

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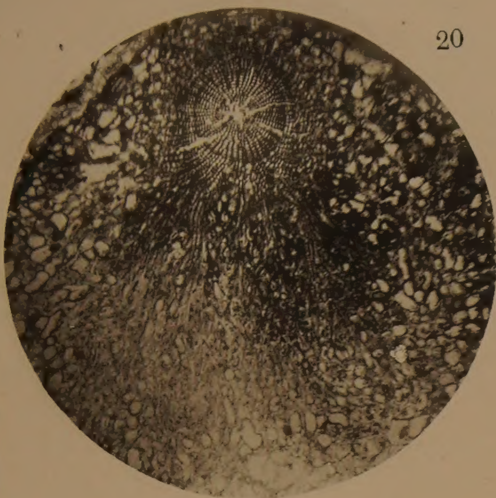
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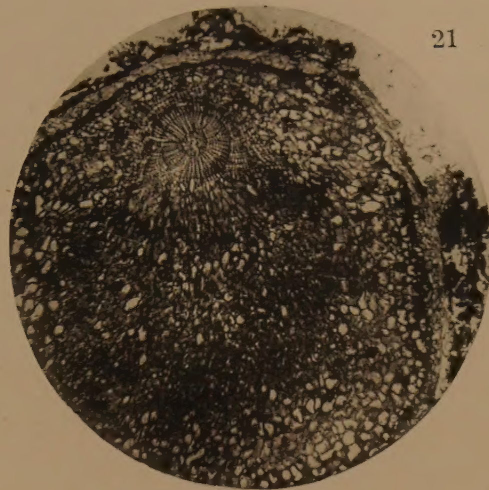
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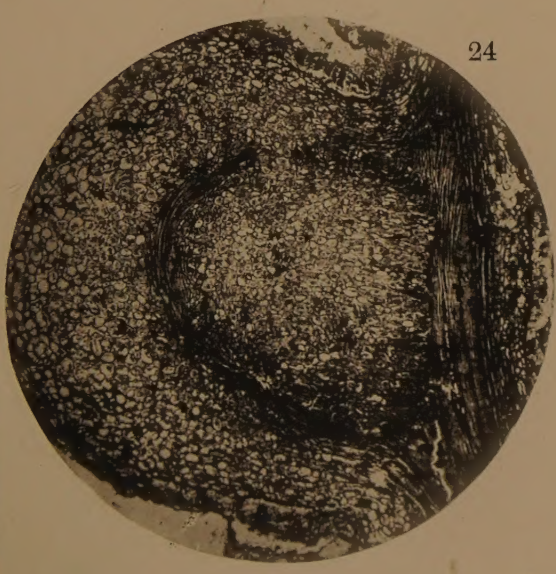
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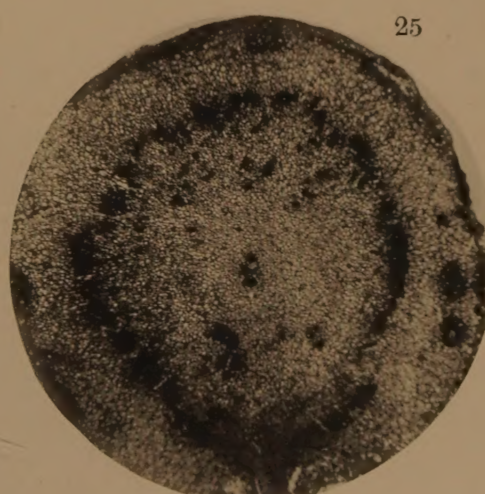
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